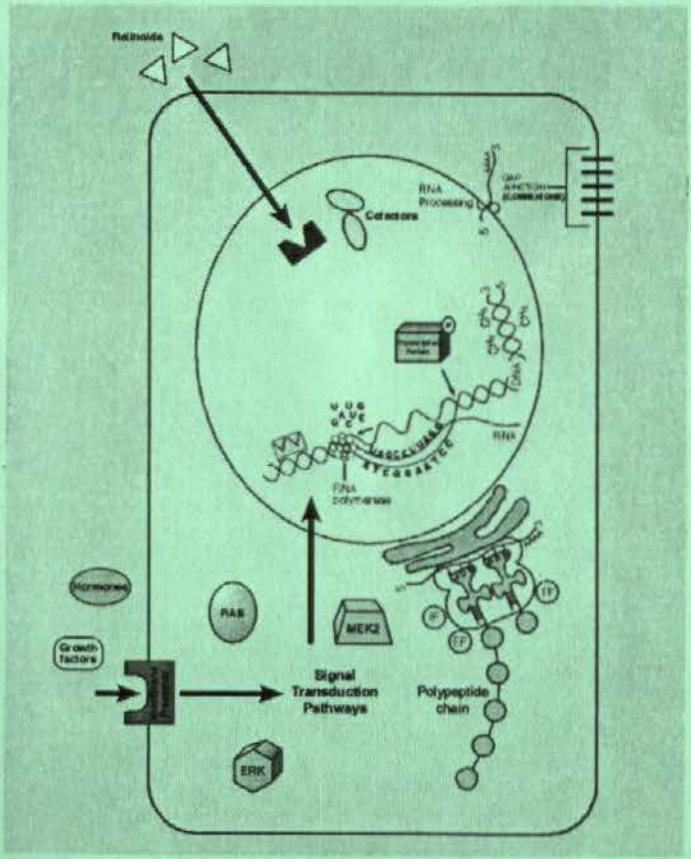
May 1, 1998

GEMS ABSTRACTS

Genotoxicity & Environmental Mutagen Society Spring Meeting

Epigenetics: a wider view of genotoxicity



Post Office Box 13475, Research Triangle Park, NC 27709

short arm
chromo 3 - lesions in alot of cancer
3p14.2 tumor suppressor gene
fragile ES L1
FHIT
can be affected by virus, chemicals
- lose FHIT

Welcome to GEMS Eleventh Spring Meeting

Epigenetics: A Wider View of Genotoxicity

May 1, 1998
Marriott Hotel, Research Triangle Park, NC

8:30 - 9:00 **Registration and Coffee**

9:00 - 9:15 **Welcome to GEMS Spring Meeting**
Dr. Elizabeth George
Introduction
Dr. Jack Bishop

9:15 - 10:15 **Overview of Epigenetic Mechanisms -**
"Genetic and Epigenetic Changes in Human Cancer"
Dr. Carlo Croce, Thomas Jefferson Universi[illegible]immel Cancer Center

10:15 - 10:45 **Break**

10:45 - 11:45 **Epigenetics in Reproductive Toxicology - [illegible]otoxicants and Reproduction: Transcriptional Resetting [illegible]e Cell Death-Susceptibility Rheostat in Germ Cells"**
Dr. Jonathan Tilly, Harvard Medical School

11:45 - 1:15 **Lunch**

1: 15- 2:15 **Epigenetics in Developmental Toxicology -**
"Toxicant Induced Phenocopies And Developmental Gene Expression"
Dr. John Rogers, US Environmental Protection Agency

2:15 - 2:45 **Break**

2:45 - 3:45 **Epigenetics in Carcinogenesis -**
"Silencing Of Genes Regulating Carcinogen Mutagenesis"
Dr. James Herman, M.D., Johns Hopkins Oncology Center

3:45 - 5:00 **Reception**

Introduction to GEMS 1998 Spring Meeting. **Jack B. Bishop,** President-Elect of GEMS, NIEHS, RTP, NC.

The topic I've chosen for this year's Spring Meeting is "Epigenetics: A wider view of Genotoxicity. Epigenetics, which is defined as 'all processes relating to the expression (transcription and translation) and the interaction of the genetic material' [Rieger, Michaelis and Green, Glossry of Genetic: Classical and Molecular, Springer-Verlag, New York, 1991], does not mean non-genetic or non-mutagenic. An agent that acts epigenetically can alter the epigenotype; which comprises the totality of interactions among genes as well as between genes and the non-genetic environment, and which produces the phenotype. The epigenotype of a cell is a stable, heritable (at least during many cell generations) character whose mode of impression is over and above, or in addition to, the classical genotype (base sequence). Epigenetic mechanisms may act at 3 levels of cell organization: (1) direct regulation of gene function, involving the turning-on and -off of genes or modulation of the synthesis of specific kinds of proteins; (2) regulation of cell differentiation by modifying the translation of RNA into proteins; and (3) regulation of the topographic distribution and function of proteins. Each of these can be affected by, among other things, changes in methylation patterns and alterations of receptors or various transcription factors. Agents can induce mutations indirectly via their epigenetic actions. But even in the absence of heritable alterations of base sequence, genetic damage involving any of the above epigenetic mechanisms can have profound effects. Damage to these genetic processes (i.e., genotoxicity) can result in cancer, reproductive failure, and abnormal development. This year's Spring Meeting highlights these epigenetic aspects of genotoxicity. Following an overview of some key epigenetic mechanisms, specific examples genetic toxicities resulting through epigenetic mechanisms or modes of action will be drawn from the areas of reproductive, developmental and cancer toxicology.

Death-susceptibility rheostat in germ cells. Jonathan L. Tilly, Ph.D.
The Vincent Center for Reproductive Biology, Department of Obstetrics and Gynecology, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts 02114.

Recent evidence indicates that germ cells, like somatic cells, possess a defined, gene-directed and evolutionarily-conserved pathway of cell death execution that is responsible for the normal depletion of gametes from the male and female gonads throughout life. Unfortunately, however, this system can be tricked into activation by a number of pathological insults, including various environmental toxicants and antineoplastic drugs. This problem in women is confounded by the fact that females of the species have a non-renewable germ cell pool, and thus irreversible sterility results from untimely death of the germline. We have begun to map these genetic events in female germ cells and have found, for example, that exposure of the female gonad to polycyclic aromatic hydrocarbons (i.e., dimethylbenzanthracene or DMBA) leads to rapid apoptosis in oocytes. This, we believe, results from DMBA-mediated activation of the aryl hydrocarbon receptor (AhR), a transcriptional regulator that is capable of inducing expression of a gene whose protein product subsequently commits oocytes to death (e.g., bax). Thus, genotoxic insults to the gonads likely carry out their "dirty work" by direct genetic alterations of key checkpoints in the programmed cell death pathway. (Supported by NIH grant R01-ES08430).

Human—
in most colon cancer cell lines
p16 hypermethylated & inactivated
RB, p16, p15 - mediation reg.
altered cell cycle control
DNA damage/repair
apoptosis
growth factor
invasion/tumor architecture

DNA mismatch repair
hMLH1 gene
Cavellieri PNAS 94: 10937-42 - 1997

Mismatch Repair Deficient
alkylating agent sens.
↑ Depurinating adducts

Epigenetics in Developmental Toxicology - Toxicant-induced phenocopies and developmental gene expression. **John M. Rogers**, Developmental Biology Branch, Reproductive Toxicology Division, NHEERL, U.S. Environmental Protection Agency.

Many malformations produced by toxicants represent phenotypes similar to those produced by "knockout" or overexpression of genes known to play important roles in development. A well-studied example of this is retinoic acid, an endogenous morphogen with a known response element in many of the homeobox (hox) genes. However, diverse xenobiotics also produce phenocopies. Knockout of hoxa-10 or hoxc-8, or overexpression of hoxc-6 produces a 14th thoracic rib in the mouse, an effect often observed in developmental toxicity studies. Overexpression of hoxa-7 results in homeotic transformations of vertebra C7 into T1 and C5 into C6, a phenotype observed in methanol-treated fetuses. Knockout of hoxa11, hoxd11 or hoxd13 results in minor limb anomalies, while knockout of both a11 and d11 causes loss of distal limb structures. Similar limb defects can be produced by several teratogens, including azacytidine. Investigators have examined changes in expression of genes associated with phenocopies induced by retinoic acid, methanol, salicylate, azacytidine and boric acid. Changes in expression of candidate hox or pax genes have been noted, but it is unclear if altered gene expression is a primary mechanism or results from other cellular alterations. Similar difficulties in interpretation arise with genes isolated by subtractive hybridization between control and treated embryos. Despite these difficulties, gene expression studies can provide insight into mechanisms of abnormal development by pointing out potential target cell populations in the embryo or helping to elucidate pathogenesis.

Silencing of genes regulating mutagensis by DNA methylation. Dr. James G. Herman, M.D., Assistant Professor, The Oncology Cent
The Johns Hopkins University, School of Medicine.

Genetic alterations are a hall mark of cancer formation and progression. Such changes involve the loss of function of tumor suppressor genes by deletion or inactivating point mutations. Recent evidence suggests that epigenetic alterations, specifically promoter region DNA methylation with associated transcriptional loss, is another important mechanism for loss of tumor suppressor gene function. While for some genes, such as p16, methylation serves as an alternative mechanism to genetic alterations for loss of gene function, the epigenetic inactivation of some genes, particularly those involved in DNA repair and carcinogen metabolism, may lead to genetic alterations in other target genes. The mismatch repair gene hMLH1 gene is epigenetically silenced in the majority of sporadic microsatellite instability positive colon and endometrial cancer, and loss of this gene function would lead to the development of mutations in genes with short repeats, ie TGF-beta receptor II and BAX. Likewise, epigenetic inactivation of MGMT may lead to point mutations in critical genes following exposure to alkylating agents, and loss of function of GST-pi may increase DNA adduct formation in certain tissue types. Such epigenetic inactivation occurs in tiss specific patterns which shed light on the role of each of these genes in the neoplastic process, and join epigenetic and genetic pathways in tumorigenesis.

Tilly

maternal exposure to cigarettes enhances oocyte death (in utero)

⊖ hormone, ↑P53 $\xrightarrow{\text{induces}}$ BAX → apop.
BAX regulated (by cytokines)
BAX knockout mouse – follicles/ don't go into apoptosis – dead cells stay there
primordial stockpile > in KO mice (BAX gone so no apoptosis)
normal # ↑ ovary age

BAX deficiency protects oocytes in vivo w/ chemo

♀ who smoke undergo menopause earlier

BAX & ...

ICH: caspase – effector molec.

welcome
Pres
welcome 11th
si students/non members
invite membership
encourage talk to member

Oct 2 Fri Sheraton
forum for student

Frank announce
e-mail address

Jack - compliment
outstanding program
record #'s in audience
Jack's efforts program
outreach

Genotoxicity & Environmental Mutagen Society

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(A non-profit organization)